labelling with iTRAQ reagents and subsequent multidimensional LC-MS/MS analysis.
Methods: 150 serum samples were obtained from OA patients at different stages of the disease (grade II, grade IV and control donors) and were grouped in pools to reduce interindividual variability. The top twenty most abundant proteins in crude serum fluids were removed by affinity chromatography using the immunodepletion column ProteoPrep® 20. Cartilage samples were obtained from OA patients undergoing joint replacement and normal donors without history of joint disease. They were extracted with Urea 6M, 2% SDS using a mixer Mill MM 200 (Retsch). Proteins were quantified, digested with trypsin and differentially labelled with isobaric tags using iTRAQ. The peptide mixture was separated by two dimensional LC coupled to MALDI-TOF/TOF mass spectrometry. Identification and relative quantification of the proteins were performed using ProteinPilot 3.0 software.
Results: Protein profiles between the OA patients (early or late) and healthy controls were compared, leading to the identification of 349 proteins. 28 proteins that were altered in OA with statistical significance and more than 1 peptide: 18 of them were increased (OA:Control >1.3, $p < 0.05$) and 10 decreased (OA:Control <0.7, $p < 0.05$). 263 proteins were identified in cartilage samples and 59 proteins were quantified with a significative p-value in OA patients compared to control. The quantified proteins of cartilage and serum are involved mainly in biological functions as extracellular organization, skeletal system development, cell adhesion, apoptosis, defence and inflammatory response. In both cases it was found that the majority of proteins belonging to the extracellular matrix region. Identification and quantification of some of these proteins both in serum and cartilage from OA patients indicate their potential biomarker value for the pathology. We detected in both samples the alteration of proteins such as cartilage oligomeric matrix protein, lumican, complement factor D or thrombospondin-1, which were found to be increased in OA, whereas the cytoplasmic actin was decreased. Finally, a number of other proteins were identified as altered either in cartilage (thus increasing our knowledge of OA pathogenesis) or in serum (which have also potential biomarker value).
Conclusions: In summary, we have identified for the first time a panel of novel OA protein biomarkers present in serum and cartilage from human patients by a proteomic approach. The specificity and selectivity of these candidates should be verified in order to develop new molecular diagnosis or prognosis tests for OA.

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DIFFERENCE GEL ELECTROPHORESIS ANALYSIS OF CARTILAGE DEGRADATION IN A MOUSE MODEL OF OSTEOARTHRITIS IDENTIFIES CHANGES IN KEY MOLECULAR PROCESSES

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Purpose: The protein profile of cartilage during the initiation and progression of osteoarthritis (OA) is likely to reflect the key early events in the disease pathogenesis. Studying OA progression using human cartilage is challenging owing to the difficulty in acquiring tissue. The surgically induced destabilisation of the medial meniscus (DMM) mouse model provides genetically homogenous cartilage at any disease stage. Proteomic comparison of diseased and control cartilage identifies protein changes occurring in early OA.
Methods: The DMM mouse model was used to study the changes in protein profile of mouse knee articular cartilage during OA. A new microdissection technique enabled targeted collection of pure mouse articular cartilage from the medial tibial plateau. Three pooled biological samples extracted from DMM and control knee joints at 2, 4 and 8 weeks were compared using difference gel electrophoresis (DIGE). Differentially expressed spots were identified by pair wise comparison of normalised spot intensities from control and OA gels. Protein identification from gel spots was performed by tandem mass spectrometry. Histological sections were used to assess cartilage degradation. Immunohistochemistry and real-time RT-PCR were used to further investigate some of the protein changes.
Results: There were no statistically significant differences between DMM and control cartilage at 2 and 4 weeks. Thirty-nine spots out of 1584 (2.5%) were statistically significantly altered in abundance at 8 weeks following DMM surgery. Thirteen distinct proteins from 26 spots were increased in DMM cartilage and 5 distinct proteins from 13 spots were decreased. Small leucine-rich repeat proteins, including biglycan, and fibromodulin, were significantly increased in both protein abundance and gene expression. Gelsolin, a regulator of the actin cytoskeleton, protein disulfide isomerase A3, fibrinogen beta chain and transitional endoplasmic reticulum ATPase were also increased. A number of proteins involved in energy production had an altered abundance; pyruvate kinase, β-enolase and aconitate hydratase were decreased in abundance, glycogen phosphorylase was increased.
Conclusions: Application of a new microdissection technique enabled analysis of the protein profile of pure mouse articular cartilage during OA progression. This technique can be used to isolate and analyse other joint tissues. The changes identified in OA cartilage at eight weeks were consistent with alterations in energy production, the cytoskeleton and cell stress. In addition, the greatly increased abundance of SLRPs adds weight to the importance of these molecules in maintaining cartilage homeostasis. Their roles extend beyond structural interactions to encompass important signalling functions.

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PHARMACOPROTEOMIC STUDY OF THE EFFECT OF THREE DIFFERENT CHONDROITIN SULFATE COMPOUNDS ON CHONDROCYTE INTRACELLULAR AND EXTRACELLULAR PROTEOMES

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Purpose: One of the most widely used compounds in the management of patients with osteoarthritis (OA) is chondroitin sulfate (CS). The aim of this work is to study drug modes of action, side-effects, and toxicity of 3 different CS compounds using two complementary proteomic approaches.
Methods: Chondrocytes were obtained from 3 OA patients undergoing joint replacement. The study was approved by the local Ethics Committee. Proteins expression analysis was carried out using DIGE (Differential In Gel Electrophoresis) and SILAC (Stable Isotope Labeling with Amino acids in Cell culture) techniques. In both cases OA chondrocytes were treated with 200 μg/mL of each brand of CS for 48 hours. For DIGE analysis, proteins samples from control and treated cells were labeled with Cy3 or Cy5 dyes, mixed by pairs and co-resolved by two-dimensional gel electrophoresis using a pool of all samples labeled with Cy2 as internal standard. Quantitative image analysis was performed using Same Spots software. The spots of interest were analyzed by mass spectrometry (MALDI-MS). Protein identification was carried out by database search (MASCOT). For secretome analysis by SILAC, cell-conditioned media from isotope-labeled (treated) and unlabeled (untrated) samples were mixed 1:1, resolved by mono-dimensional gel electrophoresis, and digested with trypsin. Separation and analysis of peptides mixtures was performed by nanoflow liquid chromatography coupled off-line to MALDI-MS. In this case protein identification and quantification was carried out with Protein Pilot software.
Results: We examined around 1500 protein spots that were present in the 6 DIGE gels. We found 46 spots differentially expressed in our conditions: 27 modulated by CS1, 4 by CS2 and 15 by CS3. We didn't observed qualitative changes in the treated cells. We could identify 28 of the altered spots, corresponding to 18 different proteins. CS1 strongly modulated mitochondrial superoxide dismutase (SOD2), a protein previously described by our group as decreased in OA cartilage. The SILAC experiment allowed us to identify 96 different proteins in the CS1 secretome, 95 in CS2 and 104 in CS3. CS1 modulated the expression of 21 proteins: 15 resulted upregulated and 6 downregulated. CS2 modulated 13 proteins: 5 upregulated and 8 downregulated. Finally CS3 modulated 9 proteins: 8 upregulated and only 1 downregulated. Each one of the studied compounds induces a characteristic protein profile in OA chondrocytes cultures. In the case of CS1 more than 60% of the altered proteins are specifically modulated only in this condition. The same occurs with CS3, while most of the proteins modulated by CS2 are in common with CS1 and CS3. Despite the highest number of proteins modulated by CS1, as revealed by DIGE and SILAC experiments, and the increased expression of SOD2, as above mentioned, some of them are catabolic or inflammatory factors like interstitial collagenase (MMP1), stromelysin-1 (MMP3) and pentraxin-related protein (PTX3).